Journal of Molecular Diagnostics, Vol. 6, No. 4, November 2004

Review

Hereditary Non-Syndromic Sensorineural Hearing Loss

Transforming Silence to Sound

Iris Schrijver

From the Department of Pathology and Pediatrics, Stanford University Medical Center, Stanford, California

Tremendous progress has been made in our understanding of the molecular basis of hearing and hearing loss. Through recent advances, we have begun to understand the fascinating biology of the auditory system and unveiled new molecular mechanisms of hearing impairment. Changes in the diagnostic impact of genetic testing have occurred, as well as exciting developments in therapeutic options. Molecular diagnosis, which is already a reality for several hearing-associated genes, will doubtlessly continue to increase in the near future, both in terms of the number of mutations tested and the spectrum of genes. Genetic analysis for hearing loss is mostly used for diagnosis and treatment, and relatively rarely for reproductive decisions, in contrast to other inherited disorders. Inherited hearing loss, however, is characterized by impressive genetic heterogeneity. An abundance of genes carry a large number of mutations, but specific mutations in a single gene may lead to syndromic or non-syndromic hearing loss. Some mutations predominate in individual ethnic groups. For clinical and laboratory diagnosticians, it is challenging to keep abreast of the unfolding discoveries. This review aims to provide the framework pertinent to diagnosticians and a practical approach to mutation analysis in the hearing impaired. *(J Mol Diagn 2004, 6:275–284)*

Hearing loss is common at all ages. It is a major public health concern because it affects 6 to 8% of the population in developed nations when all causes are combined and it is the most common birth defect.[1] Following the implementation of universal newborn hearing screening (UNHS) the incidence was found to be even higher than previously thought. Approximately one in 1000 newborns are deaf, one in 300 children are affected with congenital hearing loss of a lesser degree, and an additional one in 1000 become profoundly hearing impaired before adulthood.[2,3] Before the implementation of early hearing detection and intervention programs, the average age at diagnosis was 1.5 to 3 years, which is well beyond the beginning of the critical interval for speech and language acquisition.[4] Undiagnosed hearing loss and diagnostic delay have a profound impact on linguistic and communicative competence, as well as cognitive and psychosocial development.[5] Delayed recognition may lead to isolation later in life.[6]

Hearing loss can be due to environmental factors, genetic defects, or a combination thereof. Presbyacusis (age-related hearing loss) is generally considered to be multi-factorial. In contrast, approximately 25% of childhood hearing impairment in the U.S. is caused by environmental factors such as prematurity, infections, exposure to ototoxic medications, and trauma. It is estimated that at least 50% of prelingual hearing loss is caused by genetic changes, whereas the etiology remains obscure in the remaining 25%. Most of these cases, however, are assumed to be of genetic origin. Thus, genetic causes account for the largest proportion of all cases of prelingual hearing loss.[7]

Hearing loss can be classified further by several criteria, including the severity (mild or 20 to 39 dB, moderate or 40 to 69 dB, severe or 70 to 89 dB, and profound or >90 dB),[1] age of onset (prelingual or post-lingual), and the physiological etiology. Conductive hearing loss is characterized by external ear anomalies or abnormalities of the ossicles in the middle ear, sensorineural hearing loss is due to inner ear malfunction, and central hearing loss is caused by defects of the VIIIth nerve, the brain stem, or the cerebral cortex. Hearing loss can also be mixed.

Accepted for publication June 7, 2004.

Address reprint requests to Iris Schrijver, M.D., Department of Pathology, L235, Stanford University Medical Center, 300 Pasteur Drive, Stanford, CA 94305. E-mail: iris.schrijver@medcenter.stanford.edu.

Genetic hearing loss has diverse etiologies and it is estimated that approximately 1% of all human genes are involved in the hearing process.[8] Leaving the patterns of inheritance aside, non-syndromic hearing loss accounts for more than 70% of all hereditary hearing loss.[9] Non-syndromic or isolated deafness is not associated with a clear pattern of other physical defects. In contrast, syndromic deafness is characterized by additional manifestations, such as retinitis pigmentosa (eg, Usher syndrome), euthyroid goiter and inner ear malformations (Pendred syndrome), craniofacial dysmorphia (Treacher-Collins syndrome), marfanoid body habitus (Stickler syndrome), renal anomalies (Alport syndrome), or the presence of long QT intervals (Jervell and Lange-Nielsen syndrome). Several hundred syndromes involving hearing loss have been described.[1] Although useful when syndromic features can be recognized, this subdivision poses a dilemma when clinical manifestations are not fully developed. This is especially common in childhood, but may also be caused by variable gene expression. For example, the goiter in autosomal recessive Pendred syndrome may develop only in adulthood, if at all. With no other symptoms evident on physical examination, the diagnosis of Pendred syndrome is likely to be missed.

Genetics

Hearing loss can follow a pattern of autosomal recessive, autosomal dominant, X-linked, and mitochondrial inheritance. The genetic basis is highly complex. Allelic mutations in some genes can cause recessive and dominant hearing loss, mutations in the same gene may cause syndromic or non-syndromic hearing loss, and recessive hearing loss may be caused by a combination of two mutations in different genes from the same functional group.

Of the estimated minimum of 50% of cases with inherited hearing loss, ~70% are non-syndromic and ~80% of these are autosomal recessive ([10], Figure 1). Non-syndromic hearing loss is most often sensorineural. It can be divided into DFNA (autosomal dominant deafness, ~15 to 20%), DFNB (autosomal recessive deafness, ~80%), DFN (X-linked deafness, ~1%), and mitochondrial deafness (at least 1%).[10,11] Autosomal dominant sensorineural hearing loss (SNHL) is often post-lingual and progressive, whereas recessive SNHL is prelingual as a rule.

Due to tremendous genetic heterogeneity, the identification of genes and gene defects that affect the process of hearing has been challenging. Considering the complexity of the auditory system, which requires interaction of a diversity of proteins including ion channels, extracellular matrix, cytoskeletal proteins, and transcription factors, this is not surprising. However, it hampers gene identification by traditional genetic methods such as the grouping of multiple families for linkage analysis. Linkage analysis is also complicated by the fact that more than one cause of hearing loss can segregate in a family after assortative mating, which is relatively common among deaf individuals.

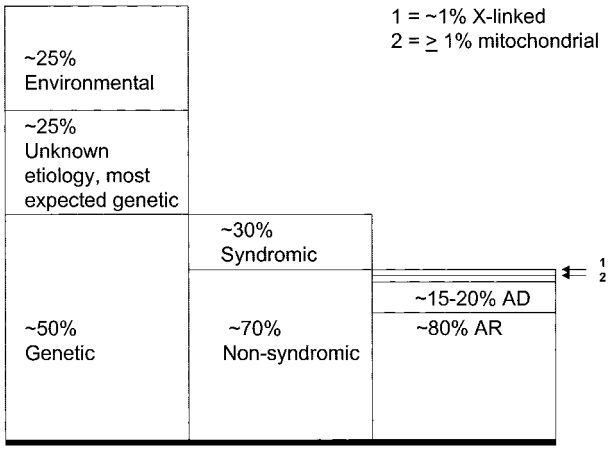

Figure 1. A subdivision of hearing impairment by cause (**column 1**), presence or absence of associated features in cases of genetic etiology (**column 2**), and mode of inheritance in the non-syndromic group (**column 3**). The **smallest boxes** in **column 3** represent X-linked hearing loss, which accounts for ~1% of non-syndromic hearing loss, and mitochondrial hearing loss, which accounts for at least 1%. AD, autosomal dominant; AR, autosomal recessive.

Genetic studies of hearing loss have been successful in isolated populations and consanguineous families. Even after the gene has been localized to a region on a chromosome, however, the process of positional cloning via a physical map followed by transcript identification can be arduous.[12] Some identified loci, including DFNB1, DFNA2, and DFNA3, have been found to harbor multiple genes. On the other hand, some genes have multiple locus assignments due to incorrect initial designations.[13] Gene identification has been facilitated greatly by the recent advances in genomics, the human fetal cochlear library, and by research in model organisms such as the mouse.[12,13]

In the field of non-syndromic hearing loss, 21 genes associated with autosomal recessive inheritance, 20 associated with autosomal dominant inheritance, and one with X-linked recessive transmission have been identified and characterized (http://dnalab-www.uia.ac.be/dnalab/hhh/).

Connexins

Autosomal recessive non-syndromic hearing loss at the DFNB1 locus on chromosome 13q11–12 is characterized by congenital, typically non-progressive, mild to profound hearing impairment. The locus contains two genes, *GJB2* and *GJB6*. *GJB2* encodes connexin 26, a gap junction protein of the beta group with a molecular weight of 26 kd. The human connexins are classified by their molecular mass (reflected by the number in the connexin name), and by extent of sequence identity, which is indicated in the gene symbols for GJA, B, and C subtypes. The connexin genes are very similar and contain their coding region within a single exon, separated from the 5′-UTR by an intron. Identification of the *GJB2* gene was a landmark in the genetics of hearing loss, because it pointed out the pivotal role of cochlear gap junction ion channels.[14] The coding sequence of *GJB2* is encompassed entirely by exon 2 and consists of 681 bp, which

are translated into a protein with 226 amino acids and include the stop codon. Exon 1 is contained in the 5′-UTR. Mutations in this gene represent the most common cause of sporadic and autosomal recessive non-syndromic sensorineural hearing loss (SNHL). They are responsible for approximately half the cases in the United States, Europe, Australia, and Israel, and have been reported in other populations as well.[15]

The most common mutation is a deletion of a single guanine from a string of six (35delG). This mutation accounts for more than two-thirds of identified mutations and results in a frame-shift with premature termination of the protein. It still remains to be determined why this mutation has a relatively high frequency, but it has been suggested that 35delG is located in a hypermutable region.[16] Both a local Chi consensus motif and a TGGGG sequence, which have been linked to β-globin gene mutations and to recombination in the immunogobulin genes, could play a role. If slippage and mispairing of strands during DNA synthesis determine the high incidence of this mutation, however, ethnic background should not contribute much to variation in frequency. Yet, the prevalence of 35delG seems to markedly vary between populations. An alternative to the mutation hot-spot hypothesis has been offered by Van Laer et al,[17] who proposed that the high frequency of this variant results from a common founder. Because the mutation is thought to be evolutionarily ancient, haplotype sharing is observed in a small chromosomal region only. Although these hypotheses seem to be contradictory, both phenomena could have contributed to the high allele frequency of this single mutation.[17] The overall carrier frequency of 35delG in the U.S. reaches 2.5% in some studies but appears to vary by population.[17–19]

The 167delT mutation is the most commonly identified mutation in the Ashkenazi Jewish population, where it has a carrier frequency of approximately 4%.[20] In Southeast Asians, 235delC is the most prevalent with a carrier frequency of ~1:100.[21] A list of all published mutations as well as common polymorphisms in *GJB2*, is available for clinical correlation and interpretation at the connexin-deafness home page (http://www.crg.es/deafness/). Approximately 110 individual non-syndromic *GJB2* gene variants have been described: eight are dominant, 89 are recessive, and 11 are of unknown significance. Reported mutations include nonsense, missense, splicing, and frame-shift mutations as well as in-frame deletions (http://www.crg.es/deafness/).

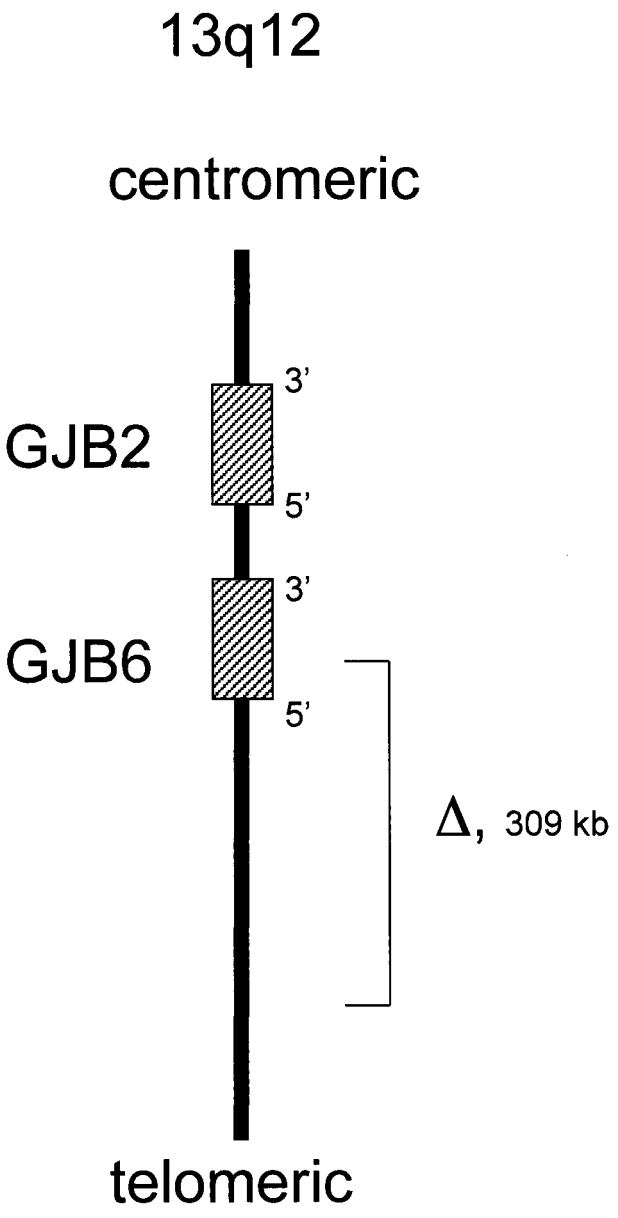

Figure 2. Schematic representation of the long arm of chromosome 13, band 12 (not to scale). The *GJB2* and *GJB6* genes, which encode connexin 26 and connexin 30, respectively, are adjacent. Δ, a 309-kb[15] deletion, which eliminates part of the *GJB6* gene and is associated with recessive hearing loss in homozygous form, or in the heterozygous state in combination with a mutation in *GJB2*. This deletion was formerly thought to span 342 kb.

Although up to half of the individuals with autosomal recessive SNHL have *GJB2* mutations, ~10 to 50% carry only one.[15] A role for *GJB6*, the gene adjacent to *GJB2* on chromosome 13, was first suggested in 1999, when a dominant mutation (T5M) was described.[22] The most common mutation in *GJB6*, however, is a >300-kb deletion which causes non-syndromic SNHL when homozygous, or when present on the opposite allele of a *GJB2* mutation.[23] *GJB2* and *GJB6* are only ~35 kb apart. *GJB2* is located on the centromeric side (Figure 2). *GJB6* is very similar to *GJB2*, but not interrupted by introns.[22] Both genes are expressed in the cochlea where they can combine to form multi-unit hemichannels in the cell membrane, and function as an integral component of the potassium regulation in the inner ear. A mouse model with connexin 30 deficiency demonstrates profound congenital hearing loss. A digenic mechanism of inheritance, therefore, is plausible. Alternatively, the large deletion could affect an undiscovered upstream regulatory element of *GJB2*.[24]

As seen with the 35delG mutation in the connexin 26 gene, the prevalence of the deletion in the connexin 30 gene seems to vary widely depending on ethnicity. The deletion frequency in study subjects with one or zero *GJB2* mutations is highest in Israel (71.4%), whereas the *GJB6* mutation occurs in up to 20% of the hearing-im-

paired U.S. population.[15] The *GJB6* deletion may account for ~10% of all DFNB1 alleles with an extremely wide range based on ethnic origin. Thus far, information is very limited and reliable estimates precluded by small sample numbers in some countries. Even within the U.S., further studies will be necessary as the overall frequency may vary markedly from state to state. Nevertheless, connexin 30 significantly contributes to the molecular basis of hearing loss. In addition to a direct effect, it is possible that the *GJB6* deletion modifies the phenotype in individuals who carry two recessive *GJB2* mutations. There can be notable intra-familial variability with hearing loss ranging from mild to profound. Thus far, however, this has not been explored.[25]

Non-syndromic dominant hearing loss associated with *GJB2* mutations is early-onset, moderate to severe, and (in contrast to autosomal recessive *GJB2* related deafness) typically progressive. Dominant *GJB2* mutations, however, often have pleiotropic effects. Hearing impairment has been reported in association with skin disorders including palmoplantar keratorderma (PPK, Vohwinkel syndrome), keratitis-ichthyosis-deafness, and hystrix-like ichthyosis deafness syndromes.[26] *GJB6* has also been associated with Clouston syndrome (hydrotic ectodermal dysplasia), which is autosomal dominant and may occur with deafness.[27,28]

Connexin Function

More than 20 widely expressed connexin proteins have been identified thus far (http://www.ncbi.nlm.nih.gov/). Through the formation of gap junctions, they enable communication between adjacent cells and are involved in a large number of cellular functions including cell growth, differentiation, reaction to signals, synchronization of activity in excitable tissues, and homeostasis.[29] The expression levels of different connexin isotypes are interconnected, as was shown in a connexin 32 knock-out mouse, which had simultaneous reduction of connexin 26 expression in hepatocytes.[30] Connexins 26, 30, 31, 32, and 43 are expressed in the inner ear where they fulfill a prominent role in normal hearing.

In the cochlea, the potassium-rich endolymph is set into wave-motion by the mechanical vibration of sound. This deflects the microvilli on the cell apices and moves the associated actin-containing stereocilia. Potassium then flows into the hair cells and depolarizes the membrane resulting in an action potential, which activates the acoustic nerve. To maintain the electrochemical potassium gradient between the hair cells and the endolymph, however, potassium ions must be recycled from the hair cells back to the endolymph.[31] Although their exact function and role in hearing loss remain unknown at present, it has been put forward that connexin gap junctions, through intercellular communication, affect the ionic ambiance of the cochlear epithelial cells and potassium recirculation.[32] Located in the cell membrane, six connexins of the same or different types form a connexon, which creates a pore in the membrane and an intercellular communication channel when aligned with a second

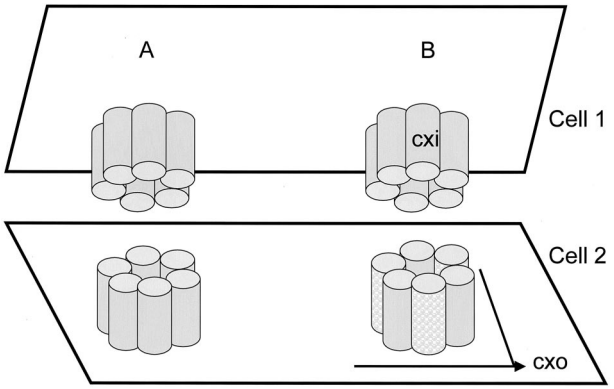

Figure 3. Six connexin proteins (cxi) group to form a hemichannel in the cell membrane. This channel is called a connexon (cxo). Connexons can be made of six identical connexins (homomeric) or of different types (heteromeric). Two neighboring connexons, which create a functional communication channel, can be identical (homotypic, as shown under **A**) or of different composition (heterotypic, as shown under **B**).

connexon in an adjacent cell[33] (Figure 3). Impairment of normal ion channel function results in hair cell death and permanent deafness.[32,34,35]

Animal studies and *in vitro* research of individual human connexins have illuminated the diverse, selective, and complex tasks in individual tissues. The multitude of cellular functions is facilitated by the expression of a variety of connexins in each cell type. Through combination of different connexins as building blocks of the individual connexons, and through formation of diverse connexon pairs, these ion channels demonstrate tremendous variety in their functions. On the other hand, connexon structure may limit certain cell-cell interactions through selective incompatibility.[29]

Mutant connexins expressed in paired *Xenopus* oocytes, with quantification of channel activity between the two cells by the dual voltage clamp technique have demonstrated a reduction or complete lack of connexon function. Whereas loss of function is the main pathogenic mechanism for recessive connexin mutations, dominant mutations exert a dominant-negative effect on the wild-type protein. The dominant-negative inhibition is most likely a result of the direct interaction of normal and defective connexins at the cell membrane.[22] It does not only occur as an effect of a defective connexin on an intact one of the same type, but it can also be *trans*-dominant, as was shown for connexin 26 and 43, which can co-localize.[36]

Connexins 26 and 30 are co-expressed in the inner ear. Connexin 30 knock-outs as well as mouse models in which connexin 26 has been selectively ablated in the inner ear (an approach chosen because connexin 26 −/− mice are not viable) have severe hearing loss, suggesting that these two connexins are unable to complement one another in the inner ear.[37] A single recessive mutation in each gene can also lead to non-syndromic SNHL through an apparently digenic mechanism. The hearing impairment could be due to a dosage effect, but one may expect that the wild-type allele of each gene would be able to compensate. Considering that there are six connexins in each connexon, however, it is likely that

Table 1. Genes Involved in Non-Syndromic Hearing Loss, for which Clinical Laboratory Testing Is Currently Available (http://www.genetests.org/servlet/access)

Chrom. location	Locus/mutation	Gene symbol	Inher.	Protein	Function	Ref.
13q11-12	DFNB1/A3	*GJB2*	AR/AD	Connexin 26	Gap junction	13
13q12		*GJB6*	AR/AD	Connexin 30	Gap junction	19
7q31	DFNB4	*SLC26A4*	AR	Pendrin	Anion transporter	38, 39, 40
14q12-13	DFNA9	*COCH*	AD	Cochlin	Extracellular matrix protein	41
Mitoch.	1555A>G 7445A>G 7472insC 7511T>C other	*MTRNR1* *MTTS1*	Mito.		12S rRNA tRNA Serine	42, 43, 44
Xq21.1	DFN3	*POU3F4*	XL	Pou domain class 3	Transcription factor	45
4p16.1	DFNA6/14/38	*WFS1*	AD	Wolframin	Not clear: ER transmembrane protein	46, 47, 48

Chrom, chromosomal; Inher., inheritance; Mitoch., mitochondrial; Ref., references.

the abnormal proteins are incorporated in the majority of heteromeric and homomeric connexons. This then results in defective connexons that cannot effectively recirculate potassium, are devoid of electric potential, and fail to provide an environment in which the hair cells survive. As yet, however, the functional basis of the digenic observation is not elucidated.

Other Genes

Several hundred genes are involved in the complex biology of hearing. Rather than to list all fully characterized genes, the following section aims to review genes other than *GJB2* and *GJB6* involved in non-syndromic hearing loss for which clinical laboratory testing is currently available (http://www.genetests.org/servlet/access) (Table 1).

SLC26A4 (DFNB4)

Mutations in *SLC26A4* are associated with autosomal recessive SNHL and with Pendred syndrome.[38,39] This is one of the most common forms of syndromic deafness, but likely under-recognized. It is associated with a dysmorphic cochlea that contains 1.5 instead of 2.5 turns (Mondini dysplasia) and with enlargement of the vestibular aquaduct, which can be examined by computer tomography. The hearing impairment is typically severe and frequently stable, although it can be progressive. The *SLC26A4* gene encodes Pendrin, a chloride/iodide transporter. Additional manifestations of Pendred syndrome include euthyroid goiter, which results from the limited ability of the thyroid gland to organify iodine during thyroid hormone biosynthesis, but this feature is neither specific nor sensitive. Goiter can be a clinical symptom of other disorders, and is not consistently present in Pendred syndrome due to incomplete penetrance. Even when expressed, it often does not manifest until adulthood. One laboratory test that may be helpful is the perchlorate discharge test, which assesses the organification of iodide. Molecular testing is valuable and feasible in this disorder, as four recurrent mutations explain ~75% of affected chromosomes. Mutations may, however, be found throughout the gene.[40]

COCH (DFNA9)

The *COCH* gene encodes cochlin, a protein abundantly expressed in the extracellular matrix of the inner ear. Hearing loss associated with defects in this gene is typically autosomal dominant, non-syndromic, post-lingual with an onset in adulthood, and progressive. Affected individuals have the unique temporal bone histopathological finding of mucopolysaccharide depositions, which appear to smother the dendritic fibers. Vestibular symptoms such as imbalance may be present. This disease has clinical similarities to Meniere's disease, but, in contrast, shows high frequency hearing loss.[41]

Mitochondrial Hearing Loss

SNHL is present in 42 to 70% of individuals with mitochondrial disorders and can be non-syndromic or syndromic. Mitochondrial DNA mutations have been identified in >3% of patients with SNHL. This figure is expected to rise due to a projected increase in genetic testing and awareness.[42] Mitochondrial hearing loss mutations are transmitted exclusively through the maternal line, and demonstrate complete or nearly complete homoplasmy, which means that the mutation is present in (almost) all of the mitochondrial genomes within an individual.[43]

Up to 25% of patients who receive aminoglycosides experience SNHL, even when administered at therapeutic levels and for a short time only. Fifty percent of those affected carry the 12S ribosomal RNA mutation 1555A>G in *MTRNR1*. SNHL could be avoided in many patients if mtDNA analysis would be performed routinely and a high index of suspicion were present before aminoglycoside administration.[42] This mutation is also an independent cause of non-syndromic, and generally milder, hearing loss in multiple ethnic backgrounds.

The 7445A>G mutation in the gene that encodes the serine tRNA causes hearing loss, but penetrance de-

pends on the on the mitochondrial haplotype in individual populations. This led to the hypothesis that either ancillary genetic modifiers or environmental factors play a role in the expression of hearing loss. Some mutations, such as a cytosine insertion at position 7472 in the tRNASer gene, are associated with sensorineural progressive hearing loss combined with neurological manifestations. Mutation 7511T>C in tRNASer is not associated with neurological manifestations, but leads to progressive hearing loss with variable age at onset.[44] More mutations have been identified in patients with non-syndromic SNHL but these are still being evaluated for pathogenicity,[43] (http://dnalab-www.uia.ac.be/dnalab/hhh/).

Mitochondrial SNHL may be syndromic and can be associated with point mutations (including those in tRNALys and tRNASer), deletions, and duplications (http://dnalab-www.uia.ac.be/dnalab/hhh/). Syndromes associated with mitochondrial hearing loss include those caused by the 3243A>G point mutation in *MTTL1*, the gene encoding the leucine tRNA. In these cases, the hearing loss exists in conjunction with Kearns-Sayre syndrome, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), maternally inherited diabetes and deafness, or chronic progressive external ophthalmoplegia.

The pathogenesis of mitochondrial hearing loss is based on the high adenosine triphosphate (ATP) requirement in the cochlear hair cells. A reduction of available ATP, caused by dysfunction of the mitochondrial oxidative phosphorylation due to mutations, results in disturbances of the ionic gradient in the inner ear. Thus, mitochondrial mutations may also contribute to the progressive hearing loss of aging, presbyacusis.[42]

POU3F4 (DFN3)

POU3F4 encodes a transcription factor (POU is an abbreviation for Pit, Oct, and Unc DNA binding domains).[45] Mutations have been found dispersed throughout the gene, with clustering in the POU domains. They are causal in X-linked, non-syndromic, progressive and profound sensorineural hearing loss (DFN3), which may demonstrate a conductive component due to stapes fixation. The stapes is a stirrup-shaped bone in the middle ear. *POU3F4* related hearing loss is another disorder for which imaging studies by CT scan may be helpful, as a widening of the internal acoustic canal, and a dilatation in the boundary between the internal acoustic canal and the inner ear may be seen. As a result, perilymphatic pressure is increased and the perilymph from the inner ear may stream out during surgical removal of the stapes.

WFS1 (DFNA6, DFNA14, DFNA38)

WFS1 was first described in 1998 by Strom et al[46] and more than 90 mutations have been identified in the *WFS1* gene since. *WFS1* encodes the glycoprotein wolframin and is associated with autosomal recessive Wolfram syndrome in the presence of inactivating mutations. It is also linked to an increased susceptibility to suicide and mental illness, as well as diabetes. Non-inactivating mutations, with a presumed dominant-negative effect, are a common cause of autosomal dominant low frequency SNHL (DFNA6/14/38).[47] The majority of these mutations are located in the carboxy-terminal protein domain. The hearing loss typically displays childhood onset and is progressive, but does not advance to profound hearing loss. The majority of patients have no significant hearing impairment in the frequencies important for speech. Thus, most do not require hearing aids.[48]

Universal Newborn Hearing Screening

In the past, a screening approach with inclusion of only high-risk infants failed to identify at least 50% of children with hearing loss, and many newborns without risk factors remained undiagnosed until after 18 months of age. If diagnosis and intervention take place before 6 months of age, however, an almost age-appropriate level of language skills can be accomplished. In 2000, the Joint Committee on Infant Hearing has endorsed Universal Newborn Hearing Screening (UNHS).[49] The aim is to provide hearing screening to all newborns before the age of 1 month, with confirmation of hearing loss in infants who do not pass the initial, or a subsequent screening, through an audiologic evaluation by the age of 3 months. Comprehensive treatment can then be initiated before the age of 6 months.[6] Most children identified through this program have parents with normal hearing.

Hearing loss can be identified by several different complementary methods. Following the guidelines from the National Institutes of Health, all U.S. states have adopted UNHS, but testing algorithms and requirements vary. The two main methods used in newborn screening are otoacoustic emission and automated auditory brain stem response.[2]

One limitation of UNHS is that not all cases of childhood hearing loss will be detected. The neonatal hearing screening may fail to identify children with progressive hearing loss, which accounts for approximately 15% of preschool children with SNHL.[11] Progression has even been described in the usually stable prelingual hearing loss associated with GJB2 mutations. In two children with homozygous 35delG mutations, the hearing loss appears to have been prelingual but not congenital. Both children passed newborn hearing tests by auditory brainstem response and a free field audiogram, respectively. This implies that an infant may pass the UNHS but could still become severely affected early in life.[50]

The UNHS test is only the first step in a successful and cost-effective program. The main goal is early diagnosis and management, normal language development, and long-term success after intervention. Data management and the tracking of infants for follow-up are currently in development. As yet, however, there is no universal protocol for medical management of hearing-impaired infants.

Medical Evaluation of Hearing Loss

The medical evaluation should begin as soon as hearing loss is suspected with a complete prenatal, medical, and family history. Risk factors such as low Apgar scores, low birth weight, respiratory distress, mechanical ventilation, admission to the neonatal ICU, hyperbilirubinemia, retrolental dysplasia, craniofacial anomalies, and chromosome abnormalities should be assessed at this time.[6]

Clinical and genetic examination are necessary to exclude the often subtle features compatible with a syndromic or congenital infectious etiology. In addition, an ophthalmology examination should be performed because ocular abnormalities are present in up to half of children with severe to profound hearing loss.[51] Every patient with unexplained hearing loss should also have a renal ultrasound and neuro-imaging of the temporal bone. Imaging studies may include a high resolution CT scan to assess the presence of a Mondini malformation, or a MRI to visualize the acoustic nerve, exclude aplasia, and rule out infectious inner ear destruction. An MRI is especially important before cochlear implant surgery.

Laboratory testing should be individualized and directed toward the suspected diagnosis. Such testing may include an IgM antibody assay in the first few years of life to assess the possibility of intrauterine infection, testing for hemoglobinopathies as these may be associated with SNHL, urinalysis and renal function tests in children with possible Alport syndrome, thyroid testing to rule out a deficiency, TSH and a perchlorate discharge test in suspected Pendred syndrome, and an evaluation of metabolic disorders such as lysosomal storage diseases.[11] An ECG to assess the QT interval should be performed if Jervell and Lange-Nielsen or Romano-Ward syndrome are suspected. Any evaluation of hearing loss requires a multidisciplinary approach, which should include counseling and support for parents. A recent survey revealed that identification of the cause of the hearing loss is the highest priority of parents who learn that their child is hearing impaired.[6]

Genetic Testing

GJB2 (connexin 26) analysis should be the first step in mutation analysis for non-syndromic sensorineural hearing loss, as it is the most common cause in its category. Samples used for testing can include peripheral blood and tissues, but also less invasive samples such as buccal cells obtained with a swab, or part of the blood spots collected for newborn screening. Both means of collecting DNA alleviate the requirement to perform venipuncture on very young children. *GJB2* mutations can be identified with a variety of allele-specific assays including PCR followed by restriction digestion, PCR with allele-specific hybridization, primer extension, or real-time PCR. Such methods are rapid, economical, and highly sensitive and specific but limiting because the number of point mutations investigated is typically low. As observed in numerous other conditions, ethnic background plays an important role in the prevalence and frequencies of mutations as illustrated by the most commonly observed mutations in Ashkenazi Jews (167delT), Asians (235delC), and Caucasians (35delG). The 35delG mutation is markedly uncommon in African Americans but the prevalence of SNHL is not. Most likely, other *GJB2* alleles such as R143W play a role. This mutation was detected in the majority of hearing-impaired study subjects in Ghana.[52] Thus, ethnic origin should be a factor in deciding which type of assay is most appropriate for a patient.

Mutation scanning methods such as DGGE, TTGE, SSCP, and DHPLC need to be fully optimized to create reproducible results. Depending on the technique, conditions, and level of optimization, some of these methods are a general mutation screen by which some mutations may be missed. Sequencing is the most comprehensive and definitive method, as almost all point mutations as well as small deletions and insertions can be detected. The relatively small size of the *GJB2* gene makes it well suited for this approach. Nearly all mutations have been detected in the coding region, which is entirely encompassed by the second exon. However, since most laboratories focus on this area only, it is not completely resolved whether the inclusion of exon one would be advantageous.[52] Drawbacks of direct DNA sequencing are the inability to detect deletions of entire exons or genes and labor intensive interpretation, although sequence comparison software facilitates this task. Analysis of a sequence in the presence of a frame-shift mutation can be challenging because other underlying mutations may be present. If a frame-shift mutation is detected, additional primers should be used to interrogate the sequence in both directions.[53] Large deletions and insertions could be investigated with a long PCR approach, coupled with gel-based analysis for sizing.

The *GJB6* deletion should be investigated as a second step in individuals in whom no, or only a single, *GJB2* mutation was detected.[54] This can, for example, be achieved by the use of primers flanking the large deletion, as this is a recurring deletion with the same breakpoints. Only individuals with the deletion would demonstrate presence of this product while a control product outside the deletion could be used for verification of amplification.[23] The *GJB2* and six products can be also targeted simultaneously by a multiplex PCR to evaluate presence of the deletion by agarose gel electrophoresis, followed by direct DNA sequencing of the open reading frame in the *GJB2* gene.[53]

In addition to UNHS and a general medical evaluation, powerful arguments can be made for genetic testing in individuals with hearing loss. 1) In the majority of individuals with genetic hearing loss, an etiology cannot be otherwise established because imaging studies are negative, extra-auditory features are not apparent, and the hearing loss phenotype does not allow categorization. The considerable and ongoing progress in our understanding of the molecular pathology of hearing impairment, conversely, increasingly enables the identification of a specific genetic defect. 2) As molecular analysis is essentially non-invasive, sedation or general anesthesia of infants and children may be avoidable and the need for more extensive, and expensive, testing may be reduced.

3) Molecular testing can result in an accurate and early diagnosis, which facilitates optimal cognitive development. 4) Molecular analysis can be beneficial for the diagnosis of syndromic hearing loss before additional features emerge (eg, in Pendred syndrome or Jervell and Lange-Nielsen syndrome), and can distinguish individuals with mitochondrial mutations who are at risk for iatrogenic hearing loss when treated with aminoglycosides. Published reports have, in general, excluded cases with clear environmental risk factors or syndromic hearing loss patients from *GJB2* analysis. Since several individuals with such risk factors and hearing loss have been shown to carry *GJB2* mutations after all, however, genetic testing may be appropriate in these cases as well. 5) *GJB2* variants may influence the expression of syndromic, environmental, progressive, and age-related hearing loss. Because *GJB2* carriers have reduced hair cell function, heterozygous mutations may contribute to more severe or earlier manifestations.[52,55] 6) Other benefits include associated knowledge of the pattern of inheritance and more accurate genetic counseling.

As more data become available, genotype-phenotype correlations can be made and the clinical significance of individual mutations, or their combinations, can be assessed more dependably. The demand for molecular tests is already increasing with the discovery of the varied molecular defects underlying hearing loss. The spectrum of molecular tests available clinically or on a research basis is also growing and can be followed on the Genetests website (http://www.genetests.org/servlet/access). It is to be expected that genetic testing will become an integral part of hearing loss evaluations at all ages, and that UNHS will be coupled with guidelines for follow-up. Even if a mutation cannot be identified at present, re-contacting and follow-up genetic testing may be warranted when additional molecular assays are developed.

Molecular diagnostic results should always be interpreted with caution, as our knowledge of the molecular basis of hearing loss is still evolving. This poses a challenge for health care providers, as few are specialized in this area. A common misperception is that a negative mutation screen rules out genetic deafness. Our reports and interpretations should be individualized, detailed, and reflect uncertainties regarding the current knowledge when appropriate. Pathogenicity has not yet been determined for all described mutations (see the connexin-deafness homepage for current connexin data, http://www.crg.es/deafness/) and new mutations are discovered by comprehensive approaches such as gene sequencing. In such cases, pathogenicity can be inferred by amino acid conservation across species, location of the mutation at a residue corresponding with pathogenic mutations in related genes, impact of a mutation on charge and polarity, coexistence with other mutations in the same gene or another, type of hearing loss identified, family studies, and an absence of the novel mutation in a large group of control subjects with the same ethnic background. Even so, pathogenicity can only be resolved by expression and functional studies.[53] Caution should also be practiced in terms of inheritance, as autosomal dominant and recessive hearing loss can be caused by mutations in the same gene. Genotype-phenotype correlations are only now emerging and are being studied on a larger scale to determine the effect of compound heterozygous mutation sets. Even with well-known mutations such as the 35delG mutation in the *GJB2* gene, however, intra-familial variability from mild to profound hearing loss can be observed and accurate prognosis is not currently possible. There may be specific missense mutations that exert an influence in the heterozygous state. Heterozygous mutations may also affect susceptibility to presbyacusis or noise-induced hearing impairment through a semi-dominant effect.[20]

Ethical Considerations

Genetic testing for hearing loss and deafness are not collectively perceived to be advantageous. Especially in the deaf community at large, deafness is neither considered to be negative, nor limiting. This community has its own linguistic culture (sign language), values and identity of which deafness is an integral part. It is not perceived to be a medical condition. Consequently, advances in hearing loss research and genetic testing might be perceived as harmful. Genetic services may be considered, however, because some individuals prefer to have deaf children. Genetic counseling services for families with deafness can only be effective and appropriate if the social values of the deaf community are taken into consideration.[56]

For hearing parents, on the other hand, having a hearing-impaired or deaf child typically raises many questions and concerns for the future. The possibility to determine the etiology of their child's hearing loss through non-invasive methods, the prospect of targeted treatment and comprehensive care early in life, together with an understanding of the mode of inheritance and chance of recurrence, are generally welcomed and lead to greater acceptance within the hearing community. Not surprisingly, prenatal diagnosis would be considered by hearing individuals more than twice as often than by deaf persons (49% versus 21%), with the hard-of-hearing falling in between (39%). Within all these groups, termination of pregnancy would be considered by a small minority only.[57]

General attitudes of the broader hearing, deaf, and hard-of-hearing community toward genetic testing were recently examined in conjunction with the widespread implementation of newborn hearing screening. Eighty-five percentage of hearing, and 62% of deaf or hard-of-hearing individuals would allow genetic analysis for their infant, which points to increasing acceptance of genetic testing for hearing loss.[58]

Treatment

Deafness is the only sensory defect that can be treated successfully even if the deafness is complete. A recent cochlear implant study in children of 8 to 9 years of age

who received their implants before the age of five, demonstrated that all children benefited from cochlear implantation in the areas of speech production, speech perception, and language. There was a significant positive difference in cognitive and reading performance in children with identified *GJB2* mutations, which cause an isolated insult to the cochlea without damage to the VIIIth nerve or the central auditory system. Even though the hearing loss in other children may be non-syndromic and isolated in appearance, the underlying etiologies are likely to include asymptomatic congenital cytomegalovirus (CMV) and undiagnosed meningitis. Thus, these children are likely to face SNHL with subtle additional disabilities due to central effects.[59]

Cochlear implant surgery has also been performed in patients with MELAS, maternally inherited diabetes and deafness, Kearns-Sayre syndrome, and chronic progressive external ophthalmoplegia. Even though a variety of mutations can cause mitochondrial hearing loss and although variable severity as well as progression after initial onset are characteristic, cochlear implant surgery has been highly beneficial. This strongly suggests that the pathological changes resulting from the mtDNA mutations primarily affect the cochlea.[42]

Conclusions

The field of hearing loss and deafness has taken great strides in the areas of research, newborn screening, molecular diagnosis, and treatment. Hearing loss is now identified early in all 50 states, and early confirmation results in the possibility of more inclusive usage of language and speech. Through an increased index of suspicion, syndromic causes can be diagnosed or excluded with a careful evaluation, and the molecular basis of deafness can be determined more reliably than ever before. In cases of non-syndromic SNHL, *GJB2* mutation analysis should always be offered, preferably in stepwise combination with *GJB6* testing. Mitochondrial inheritance and testing should be considered in any family with multiple affected individuals, except when the hearing loss was clearly transmitted through a male. When more assays become available, molecular testing could become the first step in the causal determination while more invasive testing may be avoided. Once cause is established, treatment such as cochlear implantation can dramatically improve communication and quality of life for many patients. At the same time, every discovery in the biology of the auditory system brings us one step closer to transforming silence to sound.

References

1. Petit C, Levilliers J, Marlin S, Hardelin J-P: Hereditary hearing loss. The Metabolic and Molecular Bases of Inherited Disease, ed 8. Edited by Scriver CR, Beaudet AL, Sly WS, Valle D. New York, McGraw-Hill, 2001, 4:6281–6328
2. Mason JA, Herrmann KR: Universal infant hearing screening by automated auditory brainstem response measurement. Pediatrics 1998, 101:221–228
3. Parving A: The need for universal neonatal hearing screening: some aspects of epidemiology and identification. Acta Paediatr Suppl 1999, 88:69–72
4. Centers for Disease Control and Prevention (CDC): Infants tested for hearing loss: United States, 1999–2001. MMWR 2003, 52:981–984
5. Yoshinaga-Itano C, Sedey AL, Coulter DK, Mehl AL: Language of early- and later-identified children with hearing loss. Pediatrics 1998, 102:1161–1171
6. Baroch KA: Universal newborn hearing screening: fine-tuning the process. Curr Opin Otolaryngol Head Neck Surg 2003, 11:424–427
7. Marres HA: Congenital abnormalities of the inner ear. Diseases of the Ear. Edited by Ludman H, Wright T. Bath, Arnold & Oxford University Press, 1998, pp 288–296
8. Friedman TB, Griffith AJ: Human nonsyndromic sensorineural deafness. Annu Rev Genomics Hum Genet 2003, 4:341–402
9. Van Camp G, Willems PJ, Smith RJ: Nonsyndromic hearing impairment: unparalleled heterogeneity. Am J Hum Genet 1997, 60: 758–764
10. ACMG: Genetics evaluation guidelines for the etiologic diagnosis of congenital hearing loss. Genetic Evaluation of Congenital Hearing Loss Expert Panel: ACMG statement. Genet Med 2002, 4:162–171
11. Hone SW, Smith RJ: Medical evaluation of pediatric hearing loss: laboratory, radiographic, and genetic testing. Otolaryngol Clin North Am 2002, 35:751–764
12. Li XC, Friedman RA: Nonsyndromic hereditary hearing loss. Otolaryngol Clin North Am 2002, 35:275–285
13. Van Laer L, Cryns K, Smith RJ, Van Camp G: Nonsyndromic hearing loss. Ear Hear 2003, 24:275–288
14. Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, Mueller RF, Leigh IM: Connexin 26 mutations in hereditary nonsyndromic sensorineural deafness. Nature 1997, 387:80–83
15. Del Castillo I, Moreno-Pelayo MA, Del Castillo FJ, Brownstein Z, Marlin S, Adina Q, Cockburn DJ, Pandya A, Siemering KR, Chamberlin GP, Ballana E, Wuyts W, Maciel-Guerra AT, Alvarez A, Villamar M, Shohat M, Abeliovich D, Dahl HH, Estivill X, Gasparini P, Hutchin T, Nance WE, Sartorato EL, Smith RJ, Van Camp G, Avraham KB, Petit C, Moreno F: Prevalence and evolutionary origins of the del(GJB6–D13S1830) mutation in the DFNB1 locus in hearing-impaired subjects: a multi-center study. Am J Hum Genet 2003, 73: 1452–1458
16. Denoyelle F, Weil D, Maw MA, Wilcox SA, Lench NJ, Allen-Powell DR, Osborn AH, Dahl HH, Middleton A, Houseman MJ, Dode C, Marlin S, Boulila-ElGaied A, Grati M, Ayadi H, BenArab S, Bitoun P, Lina-Granade G, Godet J, Mustapha M, Loiselet J, El-Zir E, Aubois A, Joannard A, Levilliers J, Garabedian E, Mueller RF, Gardner RJM, Petit C: Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene. Hum Mol Genet 1997, 6:2173–2177
17. Van Laer L, Coucke P, Mueller RF, Caethoven G, Flothmann K, Prasad SD, Chamberlin GP, Houseman M, Taylor GR, Van de Heyning CM, Fransen E, Rowland J, Cucci RA, Smith RJ, Van Camp G: A common founder for the 35delG GJB2 gene mutation in connexin 26 hearing impairment. J Med Genet 2001, 38:515–518
18. Green GE, Scott DA, McDonald JM, Woodworth GG, Sheffield VC, Smith RJ: Carrier rates in the midwestern United States for GJB2 mutations causing inherited deafness. JAMA 1999, 281:2211–2216
19. Fitzgerald T, Duva S, Ostrer H, Pass K, Oddoux C, Ruben R, Caggana M: The frequency of GJB2 and GJB6 mutations in the New York State newborn population: feasibility of genetic screening for hearing defects. Clin Genet 2004, 65:338–342
20. Cohn ES, Kelley PM: Clinical phenotype and mutations in connexin 26 (DFNB1/GJB2), the most common cause of childhood hearing loss. Am J Med Genet 1999, 89:130–136
21. Yan D, Park HJ, Ouyang XM, Pandya A, Doi K, Erdenetungalag R, Du LL, Matsushiro N, Nance WE, Griffith AJ, Liu XZ: Evidence of a founder effect for the 235delC mutation of GJB2 (connexin 26) in east Asians. Hum Genet 2003, 114:44–50
22. Grifa A, Wagner CA, D'Ambrosio L, Melchionda S, Bernardi F, Lopez-Bigas N, Rabionet R, Arbones M, Monica MD, Estivill X, Zelante L, Lang F, Gasparini P: Mutations in GJB6 cause nonsyndromic autosomal dominant deafness at DFNA3 locus. Nat Genet 1999, 23:16–18
23. Del Castillo I, Villamar M, Moreno-Pelayo MA, del Castillo FJ, Alvarez A, Telleria D, Menendez I, Moreno F: A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. N Engl J Med 2002, 346:243–249

24. Wu BL, Kenna M, Lip V, Irons M, Platt O: Use of a multiplex PCR/sequencing strategy to detect both connexin 30 (GJB6) 342 kb deletion and connexin 26 (GJB2) mutations in cases of childhood deafness. Am J Med Genet 2003, 121A:102–108
25. Stevenson VA, Ito M, Milunsky JM: Connexin-30 deletion analysis in connexin-26 heterozygotes. Genet Test 2003, 7:151–154
26. Brown CW, Levy ML, Flaitz CM, Reid BS, Manolidis S, Hebert AA, Bender MM, Heilstedt HA, Plunkett KS, Fang P, Roa BB, Chung P, Tang HY, Richard G, Alford RL: A novel GJB2 (connexin 26) mutation, F142L, in a patient with unusual mucocutaneous findings and deafness. J Invest Dermatol 2003, 121:1221–1223
27. Lamartine J, Munhoz Essenfelder G, Kibar Z, Lanneluc I, Callouet E, Laoudj D, Lemaitre G, Hand C, Hayflick SJ, Zonana J, Antonarakis S, Radhakrishna U, Kelsell DP, Christianson AL, Pitaval A, Der Kaloustian V, Fraser C, Blanchet-Bardon C, Rouleau GA, Waksman G: Mutations in GJB6 cause hidrotic ectodermal dysplasia. Nat Genet 2000, 26:142–144
28. Smith FJ, Morley SM, McLean WH: A novel connexin 30 mutation in Clouston syndrome. J Invest Dermatol 2002, 118:530–532
29. Richard G: Connexin gene pathology. Clin Exp Dermatol 2003, 28: 397–409
30. Simon AM, Goodenough DA: Diverse functions of vertebrate gap junctions. Trends Cell Biol 1998, 8:477–483
31. Battey JR JF: Using genetics to understand auditory function and improve diagnosis. Ear Hear 2003, 24:266–269
32. Saez JC, Berthoud VM, Branes MC, Martinez AD, Beyer EC: Plasma membrane channels formed by connexins: their regulation and functions. Physiol Rev 2003, 83:1359–3400
33. Goodenough DA, Goliger JA, Paul DL: Connexins, connexons, and intercellular communication. Annu Rev Biochem 1996, 65:475–502
34. Rabionet R, Gasparini P, Estivill X: Molecular genetics of hearing impairment due to mutations in gap junction genes encoding beta connexins. Hum Mutat 2000, 16:190–202
35. Teubner B, Michel V, Pesch J, Lautermann J, Cohen-Salmon M, Sohl G, Jahnke K, Winterhager E, Herberhold C, Hardelin JP, Petit C, Willecke K: Connexin30 (Gjb6)-deficiency causes severe hearing impairment and lack of endocochlear potential. Hum Mol Genet 2003, 12:13–21
36. Rouan F, White TW, Brown N, Taylor AM, Lucke TW, Paul DL, Munro CS, Uitto J, Hodgins MB, Richard G: Trans-dominant inhibition of connexin-43 by mutant connexin-26: implications for dominant connexin disorders affecting epidermal differentiation. J Cell Sci 2001, 114:2105–2113
37. Marziano NK, Casalotti SO, Portelli AE, Becker DL, Forge A: Mutations in the gene for connexin 26 (GJB2) that cause hearing loss have a dominant-negative effect on connexin 30. Hum Mol Genet 2003, 12:805–812
38. Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, Adawi F, Hazani E, Nassir E, Baxevanis AD, Sheffield VC, Green ED: Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). Nat Genet 1997, 17:411–422
39. Li XC, Everett LA, Lalwani AK, Desmukh D, Friedman TB, Green ED, Wilcox ER: A mutation in PDS causes non-syndromic recessive deafness. Nat Genet 1998, 18:215–217
40. Reardon W, Coffey R, Phelps PD, Luxon LM, Stephens D, Kendall-Taylor P, Britton KE, Grossman A, Trembath R: Pendred syndrome: 100 years of under-ascertainment? Q J Med 1997, 90:443–447
41. Robertson NG, Lu L, Heller S, Merchant SN, Eavey RD, McKenna M, Nadol Jr JB, Miyamoto RT, Linthicum Jr FH, Lubianca Neto JF, Hudspeth AJ, Seidman CE, Morton CC, Seidman JG: Mutations in a novel cochlear gene cause DFNA9, a human nonsyndromic deafness with vestibular dysfunction. Nat Genet 1998, 20:299–303
42. Sinnathuray AR, Raut V, Awa A, Magee A, Toner JG: A review of cochlear implantation in mitochondrial sensorineural hearing loss. Otol Neurotol 2003, 24:418–426
43. Fischel-Ghodsian N: Mitochondrial deafness. Ear Hear 2003, 24:303–313
44. Sue CM, Tanji K, Hadjigeorgiou G, Andreu AL, Nishino I, Krishna S, Bruno C, Hirano M, Shanske S, Bonilla E, Fischel-Ghodsian N, DiMauro S, Friedman R: Maternally inherited hearing loss in a large kindred with a novel T7511C mutation in the mitochondrial DNA tRNA(Ser(UCN)) gene. Neurology 1999, 52:1905–1908
45. Bitner-Glindzicz M, Turnpenny P, Hoglund P, Kaariainen H, Sankila EM, van der Maarel SM, de Kok YJ, Ropers HH, Cremers FP, Pembrey M, Malcolm S: Further mutations in Brain 4 (POU3F4) clarify the phenotype in the X-linked deafness, DFN3. Hum Mol Genet 1995, 4:1467–1469
46. Strom TM, Hortnagel K, Hofmann S, Gekeler F, Scharfe C, Rabl W, Gerbitz KD, Meitinger T: Diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (DIDMOAD) caused by mutations in a novel gene (wolframin) coding for a predicted transmembrane protein. Hum Mol Genet 1998, 7:2021–2028
47. Bespalova IN, Van Camp G, Bom SJ, Brown DJ, Cryns K, DeWan AT, Erson AE, Flothmann K, Kunst HP, Kurnool P, Sivakumaran TA, Cremers CW, Leal SM, Burmeister M, Lesperance MM: Mutations in the Wolfram syndrome 1 gene (WFS1) are a common cause of low frequency sensorineural hearing loss. Hum Mol Genet 2001, 10: 2501–2508
48. Cryns K, Sivakumaran TA, Van den Ouweland JM, Pennings RJ, Cremers CW, Flothmann K, Young TL, Smith RJ, Lesperance MM, Van Camp G: Mutational spectrum of the WFS1 gene in Wolfram syndrome, nonsyndromic hearing impairment, diabetes mellitus, and psychiatric disease. Hum Mutat 2003, 22:275–287
49. Guilford P, Ben Arab S, Blanchard S, Levilliers J, Weissenbach J, Belkahia A: Joint Committee on Infant Hearing; American Academy of Audiology; American Academy of Pediatrics; American Speech-Language-Hearing Association; Directors of Speech and Hearing Programs in State Health and Welfare Agencies. Year 2000 position statement: principles and guidelines for early hearing detection and intervention programs. Pediatrics 2000, 106:798–817
50. Green GE, Smith RJ, Bent JP, Cohn ES: Genetic testing to identify deaf newborns. JAMA 2000, 284:1245
51. Armitage IM, Burke JP, Buffin JT: Visual impairment in severe and profound sensorineural deafness. Arch Dis Child 1995, 73:53–56
52. Kenneson A, Van Naarden Braun K, Boyle C: GJB2 (connexin 26) variants and nonsyndromic sensorineural hearing loss: a HuGE review. Genet Med 2002, 4:258–274
53. Wu BL, Lindeman N, Lip V, Adams A, Amato RS, Cox G, Irons M, Kenna M, Korf B, Raisen J, Platt O: Effectiveness of sequencing connexin 26 (GJB2) in cases of familial or sporadic childhood deafness referred for molecular diagnostic testing. Genet Med 2002, 4:279–288
54. Pandya A, Arnos KS, Xia XJ, Welch KO, Blanton SH, Friedman TB, Garcia Sanchez G, Liu MD XZ, Morell R, Nance WE: Frequency and distribution of GJB2 (connexin 26) and GJB6 (connexin 30) mutations in a large North American repository of deaf probands. Genet Med 2003, 5:295–303
55. Steel KP: A new era in the genetics of deafness. N Engl J Med 1998, 339:1545–1547
56. Middleton A, Hewison J, Mueller RF: Attitudes of deaf adults toward genetic testing for hereditary deafness. Am J Hum Genet 1998, 63:1175–1180
57. Middleton A, Hewison J, Mueller R: Prenatal diagnosis for inherited deafness: what is the potential demand? J Genet Couns 2001, 10: 121–131
58. Martinez A, Linden J, Schimmenti LA, Palmer CG: Attitudes of the broader hearing, deaf, and hard-of-hearing community toward genetic testing for deafness. Genet Med 2003, 5:106–112
59. Bauer PW, Geers AE, Brenner C, Moog JS, Smith RJ: The effect of GJB2 allele variants on performance after cochlear implantation. Laryngoscope 2003, 113:2135–140